THE FOOD-VACUOLE IN THE PERITRICHA, WITH SPECIAL REFERENCE TO THE HYDROGEN-ION CONCENTRATION OF ITS CONTENT AND OF THE CYTOPLASM

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Introduction

The food-vacuole in the Peritricha was probably first seen in *Vorticella* sp. by Ehrenberg in 1830. Since then it has been observed in many different species by various investigators but it has not been intensively studied in any. There are consequently a number of unsolved problems concerning it. Some of these problems are considered in the following pages.

Material

Observations were made on the following species: *Epistylis plicatilis* (Ehrenberg), *Campanella umbellaria* (Linnaeus), *Campanella tincta* (Stokes), *Vorticella microstoma* (Ehrenberg), *Vorticella similis* (Stokes), *Vorticella convallaria* (Linnaeus), *Vorticella campanula* (Ehrenberg), *Carchesium epistylis* (Claparede and Lachmann), *Zoothamnium arbuscula* (Ehrenberg) and *Ophrydium ectatum* (Mast, 1944).

It was found that the internal structure can be more clearly seen in *Vorticella similis* and *Campanella umbellaria* and *tincta,* than in any of the other species. These three species were consequently much more thoroughly studied than the others. The specimens of *Campanella tincta* used were collected by Dr. A. Dawson in a pond containing much *Elodea* in the vicinity of New York City and shipped to Woods Hole where they lived well in the laboratory for more than a

week. Nearly all were attached to the stems and leaves of *Elodea* and most of them were single. *Campanella umbellaria* was found in abundance in a shallow ditch in a peat marsh adjoining a small lake known as Sol's pond about one mile northeast of Falmouth, Mass. The water in this ditch was covered with duckweeds (*Lemna*) and was distinctly acid (pH 6.2) but clear. Ehrenberg (1838) and Greeff (1870–71) called this organism *Epistylis flavicans*.

The two species of *Campanella* studied were practically the same in form, gross structure, size, formation of colonies and behavior, but the former had six double rows of cilia on the peristome, was grayish in color, owing to numerous conspicuous granules, and was found chiefly on *Elodea*, while the latter had only four double rows of cilia, was distinctly yellowish in color, had no conspicuous granules, and was found chiefly on *Lemna*.

Vorticella similis was found in abundance attached to duck-weeds in a pond which contained all sorts of refuse including much ashes. The water in this pond was continuously distinctly alkaline, usually pH 8.2. Most of the specimens used were, however, obtained from laboratory cultures. They thrive indefinitely in boiled tap-water containing crushed hemp seeds (two seeds in 50 cc. in a finger bowl) if the solution is renewed about once a week. In the pond they were always found very near the surface and in the laboratory they grew well only in shallow water. They apparently require an abundance of oxygen.

Structure of the Feeding Apparatus

Introduction

All observers agree that the feeding apparatus in the Peritricha contains a ciliated tube which is connected with the peristome, that this tube consists of an outer part in which the cilia produce an ingoing and an outgoing current and an inner part in which they produce only an ingoing current, and that the fecal substance and the content of the contractile vacuole are discharged into the outer part. There is, however, much variation in the names applied to these two parts and great diversity of opinion concerning the structure of the cytoplasm beyond the distal end of the inner part, as set forth in the following paragraphs.

The outer part is called "buccal cavity" by some, "vestibulum" by others and "vestibule" by still others. The outer opening of this part is called "mouth" by some, and the inner opening is called "mouth" by others. The inner part is called "pharynx" by some, "cytopharynx" by others and "oesophagus" or gullet by still others. Some hold that it opens directly into the cytoplasm, others that it does not. We shall call the outer part "vestibulum," the inner part "pharynx," and the outer opening of the vestibulum "mouth."

Ehrenberg (1838) concludes, on the basis of the direction of the movement of the food-vacuoles through the cytoplasm, that the pharynx opens into a tube or gut which extends through the cytoplasm to the anus in the wall of the vestibulum. Koehring (1930, p. 55) supports this conclusion. She could not see a differentiated tube in the cytoplasm but she says that the "orderly course" of food-vacuoles in *Vorticella* sp., and other evidence, indicates that there is a "digestive system in ciliates, comparable to the digestive system of many metazoan organisms." Greeff (1870) could find no evidence of a digestive system in the peritricha but he main-

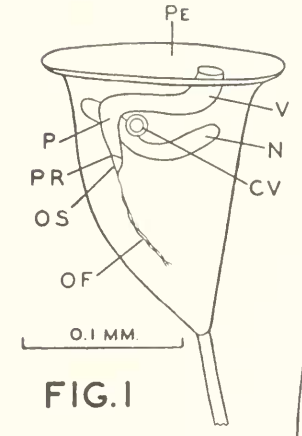

FIG. 1

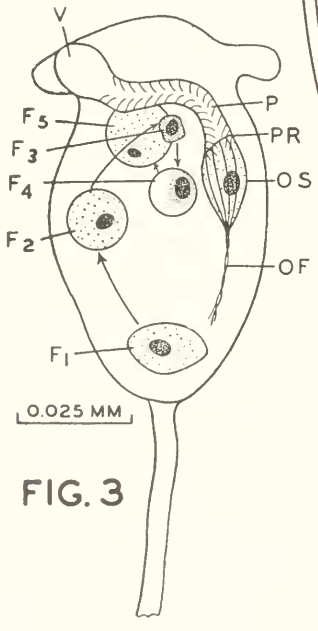

FIG. 3

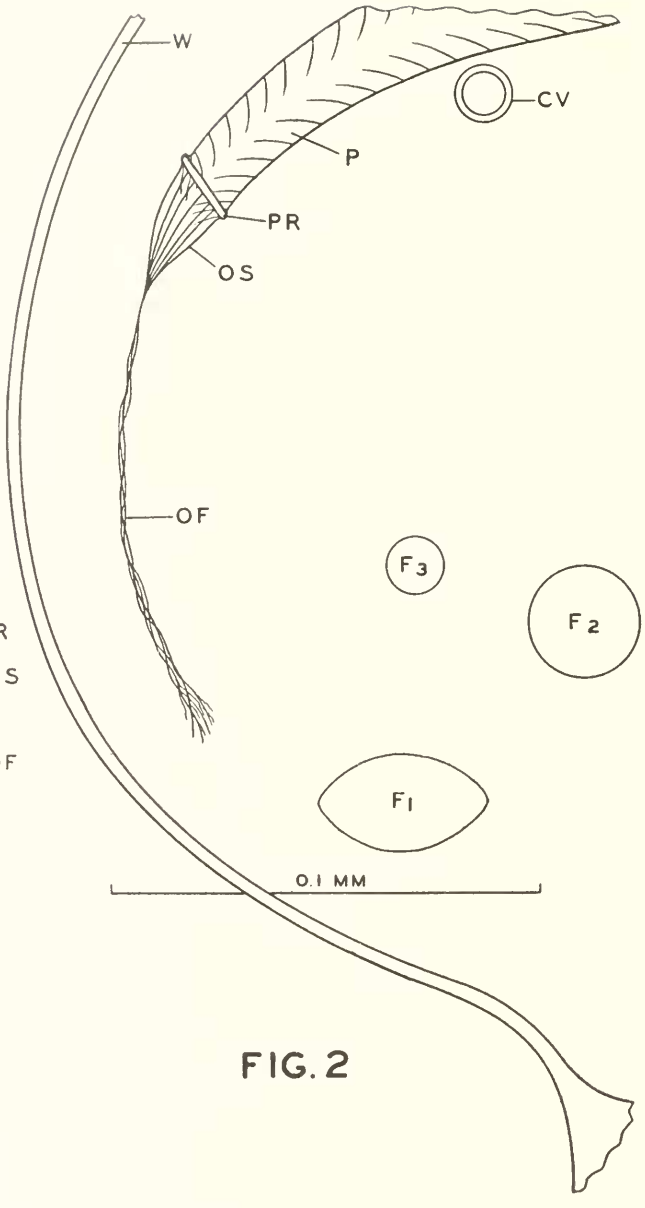

FIG. 2

FIGURE 1. Camera outline of *Campanella umbellaria*. *Pe*, peristome containing six double rows of cilia, not shown; *V*, Vestibulum; *P*, pharynx; *PR*, pharyngeal ring; *OS*, oesophageal sac; *OF*, oesophageal fibers; *N*, nucleus; *CV*, contractile vacuole.

FIGURE 2. Camera sketch of a portion of the feeding apparatus in *Campanella umbellaria* greatly compressed. *P*, pharynx; *PR*, pharyngeal ring; *OS*, oesophageal sac; *OF*, oesophageal

tains that in at least some of them (*Campanella umbellaria*) the pharynx opens into a funnel-shaped structure ("der Trichter") which in turn opens into a tube ("Oesophagus") but that this tube opens directly into the cytoplasm near the posterior end of the body, not into the vestibulum near the anterior end. Schröder (1906) says he observed such a tube in *Epistylis plicatilis* and *Vorticella monilata* as well as in *Campanella umbellaria* and Kahl (1935) concludes that it is present in all the Peritricha. Greenwood (1894) and Kitching (1938) were however unable to find any indication of it in any of some ten species studied.

Material and methods

Observations were made on the structure of the feeding apparatus in all the species listed above but certain parts of it could be more clearly seen in the two species of *Campanella* than in any of the others. No difference was found in the feeding apparatus in these two species. They were consequently used indiscriminately. Both contain so much opaque substance that their internal structure cannot be made out under normal conditions. It was found, however, that, owing to their tough elastic surface membrane, they can be greatly compressed without injury and that this greatly facilitates observations on their structure.

The observations were made as follows: A small unattached colony in tap-water containing a little powdered carmine, was mounted under a cover-glass supported by two small parallel ridges of vaseline. Water was then very slowly removed with a strip of filter paper until the campanellae were compressed as much as desired. During this process they were closely observed under low and high magnification. In some preparations the organisms were fixed by drawing Schaudinn fluid and alcohol under the cover-glass. However, this did not facilitate observations on the structure. Compensating oculars (10, 15 and 20x), apochromatic objectives (10, 20 and 40x dry and 60x oil immersion, n. ap. 1.4), an achromatic condenser and a concentrated filament lamp, with ground glass ray-filter and an iris diaphragm, were used in all the observations.

Results

The results obtained are presented in Figures 1 and 2 and the following paragraphs: These figures show that the wall of the pharynx in *Campanella* is considerably thicker at the distal end than elsewhere and that attached to this end there are several fibers which converge as they proceed and soon form a bundle which extends through the cytoplasm nearly to the posterior end of the body. The thickened end of the wall of the pharynx forms a definite ring which is highly refractive and distinctly yellowish in color. We have designated it the pharyngeal ring and the fibers attached to it, the oesophageal fibers (Fig. 2). The oesophageal fibers can be seen near the ring only under occasional circumstances and then not

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fibers; *CV*, contractile vacuole; F_1-F_3, food-vacuoles, showing change in shape and size; *W*, membrane at the surface of the body.

FIGURE 3. Camera outline of an optical section of *Vorticella similis*. *V*. vestibulum; *P*, pharynx; *PR*, pharyngeal ring; *OS*, oesophageal sac; *OF*, oesophageal fibers; F_1-F_5, food vacuoles, showing change in form and size; small dots, bacteria and granules; large dots, yeast-cells (The body contains numerous food-vacuoles and granules not represented).

very distinctly. They can however be seen definitely in the bundle but they cannot be clearly differentiated because they are superimposed and close together. Seven were definitely seen in one bundle and three to five in others. There probably are a few more than seven and they probably are equally spaced in their attachment to the pharyngeal ring. In some specimens the bundle was spread out considerably at the end forming a brush. In specimens which have been compressed and killed under a cover-glass the oesophageal fibers remain intact for several days if the preparation is sealed with vaseline and kept in a damp chamber and they do not decrease appreciably in distinctness for at least two days.

No activity was seen in the oesophageal fibers except in one specimen. This specimen was greatly compressed. The cytoplasm in it had gathered around an irregular cavity at the end of the pharynx. The cilia in the pharynx were still active and were forcing fluid into this cavity, which was abnormally large so that the oesopageal fibers were much distorted in their arrangement and in their connection with the pharyngeal ring. Three of these fibers, only slightly separated from each other, extended through this cavity near one side and then joined the rest in the bundle. Waves were definitely seen to pass synchronously along these three fibers, from their attachment to the ring, on into the bundle. This activity continued, however, only a few moments after which the entire organism appeared to be dead.

Numerous attempts were made to reproduce the conditions under which this was seen but without success. In one specimen, however, in which an irregular cavity had formed at the end of the pharynx, six inactive oesophageal fibers were seen to extend from the pharyngeal ring through the cavity. The physiological state necessary for activity in these fibers probably continues such a short time after the campanellae are compressed that it is rarely encountered.

Oesophageal fibers were seen in all the other species studied and a pharyngeal ring in several. The fibers were fairly distinct in *Vorticella similis* (Fig. 3), *Epistylis plicatilis* and *Ophrydium ectatum* (Mast, 1944), but they could not be counted with certainty in any of them, although seven were distinctly seen in one ophrydium and five in one vorticella. There doubtless are more, probably about ten.

Numerous specimens of *Vorticella similis* were fixed (some in hot Schaudinn and others in hot Bouin fluid) stained with Heidenhain haematoxylin, and sectioned (3, 5, 7 and 10μ). Those fixed in Bouin fluid were much better than those fixed in Schaudinn, but the oesophageal fibers could not be as distinctly seen in either as in living specimens.

There was no indication of an oesophageal tube in any of the species studied. If there actually is such a tube the fibers observed must be in its wall. There is, however, considerable evidence (presented later) which opposes this supposition. There is, then, in the results obtained no support for the views of Ehrenberg and Koehring or Greeff and Schröder presented above.

Fibers extending from the pharynx have been seen by Schuberg (1890) in *Stentor,* Sharp (1914) in *Diplodinium,* Andrews (1923) in *Folliculina* and Bozler (1924) and Lund (1941) in *Paramecium.* Schuberg and Andrews maintain that the fibers are in the wall of an oesophageal tube. Sharp, Bozler and Lund maintain that they extend directly through the cytoplasm. The views concerning their function vary greatly.

Formation and Movement of the Food-vacuoles

It is well known that in the peritricha the food-particles aggregate at the distal end of the pharynx, but opinions differ as to how the food-vacuoles are formed and transported through the cytoplasm.

Numerous observations were made on the process of feeding in many specimens of *Campanella umbellaria* and *tincta* and *Vorticella similis* under various conditions, and on a few specimens of each of the other species listed above. The results obtained in the observations on *Campanella* led to the following conclusions:

When the organisms are not feeding there is at the distal end of the pharynx a cone-shaped space filled with culture fluid and particles suspended in it. At the surface of this space there is a membrane in the form of a cone-shaped sac which we shall call the oesophageal sac (Fig. 2). This membrane is doubtless produced by the interaction between the fluid in the space and the adjoining cytoplasm. Pharyngeal cilia project into the sac and the oesophageal fibers pass from the pharyngeal ring over its surface to its apex where they unite to form a bundle which passes on into the cytoplasm. When feeding begins the pharyngeal cilia force more culture fluid and particles into the oesophageal sac. This stretches the membrane around it, but continuous interaction between the fluid in it and the adjoining cytoplasm prevents this membrane from becoming too thin. As the sac enlarges it becomes spindle-shaped, owing to unequal pressure of the oesophageal fibers and possibly the adjoining cytoplasm on different regions of its surface. Under normal conditions enlargement continues until the sac is nearly twice as wide as the pharynx, then a constriction begins to form near the pharyngeal ring. This constriction increases until a spindle-shaped portion of the sac is pinched off, leaving a cone-shaped portion attached to the pharynx, the same in shape and size as that which obtained before feeding began (Fig. 4 *A–E*). The spindle-shaped portion is a new food-vacuole. There is no perceptible change in size of the pharynx or the pharyngeal ring during this process. These structures are consequently not directly involved in the formation of the food-vacuole.

The newly formed food-vacuole moves rapidly through the cytoplasm to the distal end of the oesophageal fibers. Here it remains a few moments, usually turning sharply, then it proceeds slowly with the cytoplasm on an indefinite course, ending in the lower part of the vestibulum where its indigestible content is discharged. Its slow movement is obviously due to the movement of the cytoplasm in which it is suspended, i.e. to cytoplasmic streaming, but during its rapid movement definite currents are produced in the adjoining cytoplasm, showing very clearly that this movement is not due to cytoplasmic streaming.

The constriction in the oesophageal sac is probably due to simultaneous inward pressure, in the same region, of the oesophageal fibers on its surface; and the food-vacuole is probably transported from the pharynx to the posterior end of the body within the bundle of oesophageal fibers by waves passing synchronously along these fibers and from the posterior end of the body to the vestibulum by streaming movement in the cytoplasm (cyclosis).

The formation and transportation of the food-vacuoles in *Vorticella similis* and all the other species studied is in full harmony with this description. In all, the food-vacuole is formed by pinching off a portion of a cone-shaped sac attached to the pharynx and in all the food-vacuole is spindle-shaped and passes rapidly

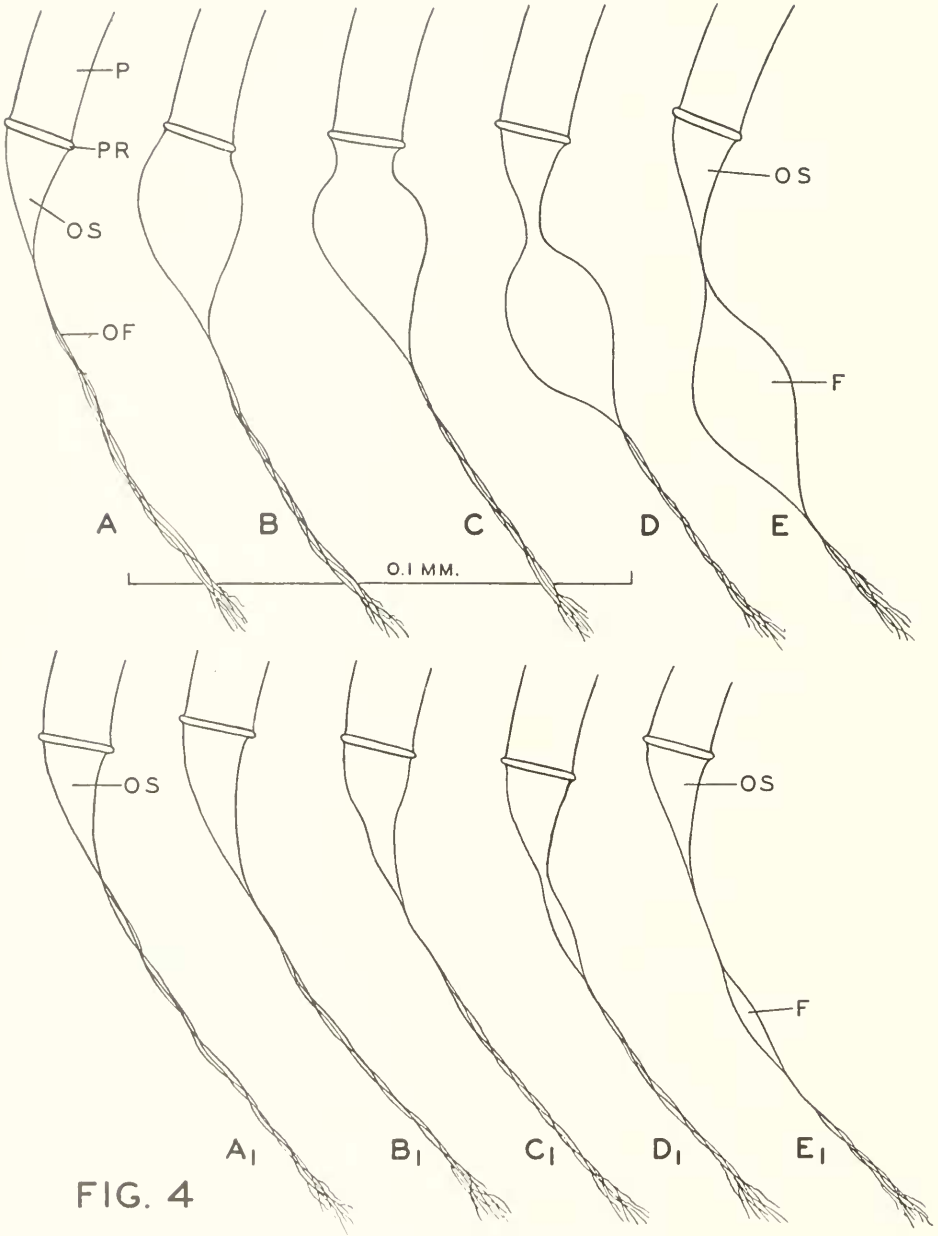

FIG. 4

FIGURE 4. Outlines showing a portion of the feeding apparatus and the formation and movement of food-vacuoles in *Campanella*. *A* and A_1, feeding apparatus in a specimen not feeding or immediately after a food-vacuole has been formed; *B-E*, successive stages in the formation of a food-vacuole under normal conditions; $B_1 - E_1$, same in a compressed individual with peristome closed and its cilia inactive. *P*, pharynx; *PR*, pharyngeal ring; *OS*, oesophageal sac; *OF*, oesophagel fibers; *F*, food-vacuole.

Note that under normal conditions the cone-shaped oesophageal sac enlarges greatly and

through the cytoplasm to the posterior end of the body and then slowly with the cytoplasm through the body. No evidence of an oesophageal tube was observed in any of these species.

These conclusions and others are strongly supported by the results obtained in detailed observations on variations in the formation and the movement of food-vacuoles in several specimens. These observations are considered in the following paragraphs:

1. In a specimen of *Vorticella similis* mounted in tap-water but not compressed, it was observed that the food-vacuoles had, immediately after they were formed, a long projection at one end. One of these vacuoles was continuously studied under the oil-immersion objective during the entire process of formation and for some time after, and the following observed:

The constriction in the oesophageal sac did not completely separate the food-vacuole from it. When the vacuole moved away this connection was drawn out until it had formed a strand fully as long as the vacuole; then it broke at the apex of the sac. The vacuole with this strand attached now moved rapidly to the posterior end of the body, then turned sharply; after which the strand folded over, came in contact with the surface of the vacuole, and fused with it; then the vacuole moved on slowly and very slowly rounded up. The membrane on the surface of this vacuole appeared to be very thick and viscous.

In other specimens of this species under the same conditions, but with powdered carmine added to the tap-water, some of the food-vacuoles remained spindle-shaped for at least one hour after they had reached the posterior end of the body and in some specimens of *Ophrydium ectatum* more than two hours, whereas they ordinarily round up in a few moments. Obviously either the membrane at the surface of these vacuoles was thicker and more viscous than ordinarily or their entire content was more viscous, probably the latter.

The results presented above show that constriction in the oesophageal sac is not the only factor involved in the formation of the food-vacuoles, that is, that in connection with this constriction there must be a mechanism which forces the vacuole toward the posterior end of the body so as to stretch out and break its connection with the oesophageal sac. They also show that the membrane at the surface of the food-vacuole is formed while it is still a part of the oesophageal sac, not after it has reached the posterior end of the body as some maintain. They show, moreover, that the membrane at the surface of the vacuole varies greatly in thickness and in viscosity and that the entire content of the vacuole probably also varies greatly in viscosity.

2. A specimen of *Campanella umbellaria* was greatly compressed and then continuously observed under the oil-immersion objective. The peristome was inverted and the cilia on it were inactive but those in the vestibulum and the pharynx were active and food-vacuoles were formed at intervals of about 45 seconds; but after nine had been given off all ciliary action ceased. All these vacuoles were spindle-shaped, but much smaller and relatively much longer than those formed under

becomes spindle-shaped, that a portion of this sac is constricted off to form the food-vacuole, and that the constriction begins at the base of the sac near the pharyngeal ring; but that under abnormal conditions the sac enlarges but little, that only a small portion is constricted off and that the constriction begins near the tip of the sac. Under both conditions the formed food-vacuole usually moves rapidly to the end of the oesophageal fibers.

normal conditions. The minor axis of the first one formed was about half as long as the diameter of the pharyngeal ring and that of the last one not more than one-sixth; whereas it usually is nearly twice as long in normal food-vacuoles. In their formation the oesophageal sac enlarged slightly, then a constriction appeared near its apex and soon a small portion of the sac was pinched off (Fig. 4 $A_1 - E_1$). This passed rapidly to the posterior end of the body then almost immediately rounded up, after which it moved slowly, decreased rapidly in size and seemed to disappear entirely. There were no visible particles in any of these food-vacuoles.

Ciliary activity in the pharynx was seen in nearly all the compressed campanellae examined, but food-vacuoles formed in only a small percentage of them. In all but a few of these the formation of food-vacuoles ceased immediately after ciliary activity in the pharynx had ceased and in these few only one vacuole formed after this.

These results indicate that the enlargement of the oesophageal sac is dependent upon activity of the cilia in the pharynx but not upon activity of those on the peristome, and they show that the formation of the food-vacuole is not specifically dependent upon ciliary action in the pharynx or the size of the oesophaeal sac or the presence of particles in suspension in the fluid in it.

3. In a compressed specimen of *Campanella tincta* five small food-vacuoles were formed in succession and rapidly transported to the posterior end of the body; then there suddenly occurred a very violent upheaval in the cytoplasm, after which a large food-vacuole was formed and transported, but very slowly and only a short distance, after which it turned sharply, nearly stopped moving and soon rounded up. Two more large vacuoles were formed after this and these also moved slowly and only a short distance, then stopped and rounded up. The large food-vacuoles were more than 20 times as large as the small ones; they moved much more slowly than the small ones and not more than half as far before they stopped and rounded up.[1]

Similar results were obtained in observations on several other compressed specimens. In one of these a very large food-vacuole formed, slowly moved back a short distance, turned sharply in its course, rounded up and stopped. Then a very small vacuole formed and moved rapidly, past the large one, nearly to the posterior end of the body after which it moved very slowly and rounded up. This was followed by the formation of two more small vacuoles, both of which moved rapidly past the large one to the posterior end of the body (Fig. 5). The large vacuole was closely observed under the oil-immersion objective. It did not move appreciably but rapidly decreased in size and disappeared entirely in three minutes.

The fact that some of these food-vacuoles went only about one-fourth as far as others before their rate of speed rapidly decreased cannot be understood on the assumption that they passed through a tube and were propelled by peristalsis in it. It can however, be readily understood on the assumption, postulated above, that their movement was due to the action of fibers which can move freely and are not fixed in their spacial interrelationship.

4. A specimen of *Vorticella similis* was mounted in tap-water and the oesophageal sac measured at maximum size. Then the tap-water was replaced by distilled

[1] The junior author asserts that in his observations on the effect of various chemicals on the size of the food-vacuoles in *Vorticella similis*, he frequently saw very small "needle-like" vacuoles form.

water and the sac measured again, after which the distilled water was replaced by 0.006 M lactose in distilled water and the sac measured once more. The averages obtained for the minor axis under these three conditions were respectively 10.5μ, 11.5μ and 12.5μ.

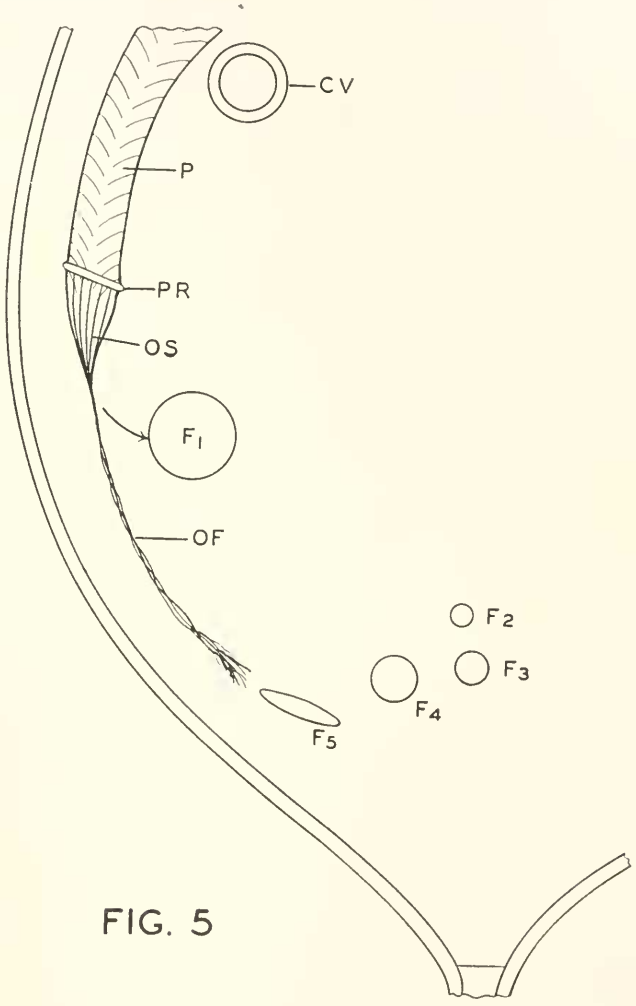

FIG. 5

FIGURE 5. Camera outline of a portion of a compressed specimen of *Campanella tincta*, showing differences in the size of successively formed food-vacuoles and difference in their direction and extent of movement.

CV, contractile vacuole; *P*, pharynx; *PR*, pharyngeal ring; *OS*, oesophageal sac; *OF*, oesophageal fibers; $F_1 - F_5$, *five* food-vacuoles formed in the order given; arrow, direction of movement.

Note that the first vacuole in this series was very much larger than the rest and moved only a short distance before it rounded up and that the four succeeding small vacuoles passed the large one and moved much further before they stopped and became spherical. F_1 was drawn very soon after it has been formed, $F_2 - F_5$ immediately after F_5 had been formed, i.e. after F_2 and F_3 had decreased considerably in size.

During one of the measurements of the oesophageal sac in the lactose solution a food-vacuole in the adjoining cytoplasm suddenly fused with the sac and caused a very marked increase in its size, immediately after which a portion of it was constricted off as an abnormally large food-vacuole. Immediately before the fusion took place the minor axis was 12.5μ long and during fusion it increased to 16.5μ.

The fact that the food-vacuoles in the cytoplasm can fuse with the oesophageal sac, strongly supports the conclusions reached above, namely that there is nothing in the nature of an oesophageal tube in these organisms and that the rapid movement of the food-vacuoles after they leave the pharynx is due to the action of a mobile structure which does not have a fixed position in the cytoplasm and does not prevent direct contact between the vacuoles and the cytoplasm.

Discussion

Greeff (1870) long ago observed that the food-vacuoles in *Campanella umbellaria* pass from the pharynx toward the posterior end of the body much more rapidly than the adjoining cytoplasm and he concluded consequently that they are not carried by the cytoplasm. He maintains, as stated above, that there is a long tube ("der Oesophagus") which extends into the cytoplasm from a spindle-shaped structure ("der Trichter") at one end of the pharynx. He asserts that in the "Trichter" the food which has been forced into it by the cilia in the pharynx, is formed into small spindle-shaped masses, which pass rapidly through the oesophagus" into the cytoplasm and that a membrane then forms at the surface of each mass and thus produces a food-vacuole. He accounts for the rapid movement of the food-vacuoles by assuming that they are forced through the "Oesophagus" by waves of contraction in it, i.e. by peristalsis.

Kahl (1935, p. 652) confirms Greeff in reference to the "oesophageal" tube in *Campanella* and concludes that such a tube is present in all the peritricha. He says: "Der Ösophagus ist bisher meist übersehen worden; er scheint aber nach eigenen Untersuchungen nie zu fehlen, ist aber nur bei grösseren Arten gut erkennenbar."

The evidence presented above indicates that Greeff is correct in his contention that at the end of the pharynx there is a funnel-shaped structure to which is attached a long narrow structure which extends into the cytoplasm, but it indicates that the latter is a bundle of fibers instead of a tube and that the former is a sac, a portion of which is separated off to form a food-vacuole, rather than a funnel in which the food is formed into spindle-shaped masses which become food-vacuoles after they have been transported through the tube into the cytoplasm. It also indicates that the food-vacuoles are propelled from the pharynx to the posterior end of the body by the action of fibers, not by contraction in the wall of a tube.

Kitching (1938, p. 87) recently observed the rapid movement of the food-vacuoles referred to above, in a considerable number of species in several genera but he found no evidence of an oesophageal tube in any of them. He concludes that the rapid movement of the vacuoles is due to waves of contraction, in accord with Greeff's contention, but that the contraction is in the cytoplasm, not in the wall of a tube in it. He says: "It is concluded that the food-vacuoles are propelled over the determined course [i.e. from the pharynx to the posterior end of the body] by contractions in the surrounding protoplasm."

It is obvious, however, that to propel a vacuole by contraction in surrounding protoplasm which is not fixed as it is in a tube, the viscosity of the protoplasm would have to be continuously lower in front of the vacuole than back of the contracting region. There is no evidence indicating that this obtains. Kitching's hypothesis consequently has no objective support.

Nirenstein (1905), Gelei (1934) and others maintain that in *Paramecium* the food-vacuole is separated from the pharynx by the pressure of protoplasmic currents. Bütschli (1889, p. 1405) and Bragg (1935, 1936) contend that contraction of the distal end of the pharynx is also involved. Lund (1941) holds that neither is involved and that the vacuole is separated from the pharynx by the action of fibers which are attached to the pharynx and extend for a considerable distance into the cytoplasm. These views will be considered in a later paper.

The Initiation of the Constriction of the Food-vacuole from the Pharynx

It is generally assumed that the initiation of the constriction of the food-vacuoles from the pharynx is correlated with the size of the enlargement at the end of the pharynx. For example, Hall and Nigrelli (1930) referring to *Vorticella* say: "After the basal portion of the gullet reaches a certain size, it is rapidly constricted from the rest of the gullet and then separated completely as a food vacuole." The fact, however, that (as demonstrated above in observations on *Campanella*) successively formed food-vacuoles sometimes vary enormously in size, shows that the initiation of their separation from the pharynx is only very superficially correlated with their size, if at all.

Bozler (1924) maintains that in *Paramecium* solid particles are necessary for the formation of food-vacuoles and that such particles must come in contact with the membrane at the end of the pharynx before a food-vacuole begins to form. Bragg (1935) maintains that while contact of a large particle with the inner surface of the "vacuolar membrane" always causes immediate separation of the food-vacuole from the pharynx, it is not necessary. We have, in observations on *Campanella* and *Vorticella,* repeatedly seen food-vacuoles form which contained no visible particles and we have seen some of these vacuoles disappear in the cytoplasm so rapidly that very little, if any, digestion could have occurred. These facts seem to show that these vacuoles contained no solid particles, and consequently that solid particles were not involved in their formation. Moreover, Schewiakoff (1891) and Wallengren (1901) assert that they observed food-vacuoles form in solutions which were free from solids.

Kitching (1938) observed that if *Pyxidinium asclli* is mounted in "1/16 to 1/8% agar" food-vacuoles form without ciliary action on the "disc" or in the "gullet." We have confirmed this in observations on Campanella. We also observed that there is no change in the size of the pharynx during the separation of the food-vacuoles from it. This separation is therefore not correlated with changes in ciliary action in the pharynx or with contraction in it.

It will be demonstrated presently that the size of the food-vacuoles depends upon the chemical composition of the surrounding medium. This seems to show that the chemical composition of the solution in the food-vacuoles has something to do with their separation from the pharynx, but it in no way accounts for the enormous variation in size referred to above, which occurred with no variation in the surrounding medium.

What is it, then, that sets off the process which separates the food-vacuoles from the pharynx?

It is highly probable that waves start at fairly regular intervals in the pharyngeal ring and pass simultaneously down all the oesophageal fibers and that each of these sets of waves initiates a constriction in the oesophageal sac, if it contains sufficient fluid to make a constriction possible. If this is true, the size of the vacuole is correlated with the rate at which fluid is forced into the oesophageal sac by the cilia in the pharynx and the rate at which it leaves this sac by osmosis. If these processes and the interval between successive waves depend upon the composition of the surrounding fluid, the temperature and the physiological state of the organism, it accounts for the observed variation in the size of the food-vacuoles and the intervals between their formation. If the food-vacuoles are separated from the pharynx by waves in the oesophageal fibers, one would, moreover, expect to find the observed correlation between the location of the constriction on the oesophageal sac and the size of the vacuole and also the observed absence of a constriction when the sac is very small. There would still remain, however, the problem of the origin of the periodic waves.

Food and Feeding

The observations considered in this and the following sections were made on *Vorticella similis* as follows:

Several small pieces of substance with vorticellae attached were mounted in pond-water or culture-fluid between two parallel ridges of vaseline on a slide. A cover-glass was then added and pressed down until the pieces of substance were much flattened, but not enough to interfere with the activities of the vorticellae. In such preparations the fluid could readily be changed as desired by applying a strip of filter paper to one edge of the cover-glass, and if the flow of fluid was continued so as to provide sufficient oxygen any selected vorticella could be studied under low or high magnification as long as desired and the effect of various substances on its activities ascertained.

Vorticella feeds almost exclusively on bacteria, but all sorts of particles in suspension in the surrounding fluid are carried into the vestibulum in the currents produced by the peristomal cilia. Many of these are, however, immediately carried out again in the outgoing current produced by the cilia in one region of the vestibulum. Nearly all the rest and some gelatinous substance secreted by the peristome or the walls of the feeding apparatus, are forced through the pharynx into the oesophageal sac by the pharyngeal cilia.[2] There is, however, great variation in the kind of particles that are selected and ingested by different individuals in the same preparation and by the same individual at different times. Yeast-cells, e.g. are, at any given time, freely ingested by some individuals and rigidly rejected by others, and freely ingested by a given individual at one time and rigidly rejected at another.

It is well known that when the food-vacuole leaves the pharynx the concentration of particles in the fluid in it is usually very much greater than it is in the fluid which enters the vestibulum. Greeff (1870) maintains that the cilia in the pharynx

[2] In *Vorticella* mounted in distilled water or in lactose (0.05 M) in tap-water or in solutions of NaCl, this gelatinous substance is very evident. It gelates as the vacuoles decrease to minimum in size (probably owing to the increase in acidity) and then solates as they increase in size. It is highly probable that it is formed under all conditions, as it appears to be in *Folliculina*, judging from the results of observations made by Andrews (1923).

come in direct contact with the particles in it and force them through the fluid into the oesophageal sac and that consequently only a relatively small amount of water is carried in with the particles. Nirenstein (1905) and Bozler (1924) referring to *Paramecium* maintain that the pharyngeal cilia force almost nothing but fluid into the oesophageal sac until it has become nearly maximum in size and then almost nothing but solid particles until it is well filled with them. Both of these views would account logically for the relatively great concentration of solid particles in the newly formed food-vacuoles. However, the results of our extensive and detailed observations do not confirm either of them.

We found that when the oesophageal sac begins to enlarge the concentration of solid particles in it is usually only slightly greater than in the fluid which enters the vestibulum, but that as the sac enlarges, the concentration of particles in it usually increases greatly.

Selective action of the pharyngeal cilia would account for the concentration of particles in the newly formed food-vacuoles, but it would not account for the observed gradual increase in concentration in the oesophageal sac except on the assumption of gradual increase in selective ciliary action. This is, however, not at all probable. How then can the gradual increase in concentration be explained?

It will be demonstrated presently that after the food-vacuole is formed fluid usually leaves it rapidly, owing to difference in osmotic concentration of the internal and external fluids. It is consequently practically certain that fluid passes continuously from the oesophageal sac out into the cytoplasm as it enlarges. The increase in the concentration of the particles in the fluid in the oesophageal sac is therefore, in all probability, due to this loss of fluid. Moreover, Frisch (1937), in observations on *Paramecium*, has demonstrated that fluid passes from the pharynx into the adjoining cytoplasm. If this obtains in *Vorticella*, it accounts for the probable increase in the concentration of solid particles as the fluid in which they are suspended passes through the pharynx.

The junior author, in his measurements of the food-vacuoles in *Vorticella* in different solutions, repeatedly saw the oesophageal sac suddenly decrease in size and at times, especially in distilled water, alternately decrease and increase like "the pumping of a heart." In one specimen in 0.014 M NaCl the oesophageal sac gradually increased to 11.56μ in diameter, then suddenly decreased to 8.84μ in diameter, then remained without further measurable change in size for 20 seconds and then left the pharynx. The decrease in size observed under these conditions was, however, doubtless due to the forcing of fluid from the oesophageal sac back into the pharynx, probably by pressure on the surface of the sac by the action of the oesophageal fibers.

The Size of the Food-vacuoles and the Time Required for their Formation

Introduction

No detailed measurements have heretofore been made on the size of the food-vacuoles in the peritricha or the time required for their formation. The results reported indicate, however, that while there is much variation in different individuals under the same conditions and in the same individual under different conditions, consecutive vacuoles do not vary much either in size or in the time required for their formation. Hall and Nigrelli (1930) imply, e.g. that in

Vorticella sp. the food-vacuoles are fairly uniform in size and the "intervals" between their formation rather constant for a given individual.

We measured many food-vacuoles and the time required for them to form in *Vorticella similis* in various solutions. Some of the results obtained will be considered in the following paragraphs. A more extended account of the work will be presented in a subsequent paper by the junior author.

Methods

Several vorticellae attached to a fragment of *Lemna,* or to a short hair, were mounted in a drop of water between two parallel ridges of vaseline on a slide and covered with a cover-glass. The slide was then put on the mechanical stage of the microscope and a narrow strip of filter paper, long enough to reach over the edge of the stage, placed at one edge of the cover-glass between the ridges of vaseline. Then some of the solution to be tested was placed on the slide at the other edge of the cover-glass between the ridges of vaseline and more added as, owing to the action of the filter paper, it flowed through under the cover-glass. A specimen which extended from its attachment well out into the current of solution was now observed. After the vorticella had been subjected to this current for ten minutes and thoroughly adapted to the new solution, measurements under an oil-immersion objective were made by means of a stopwatch and an ocular micrometer, on a series of successive food-vacuoles, in reference to the time required for their formation and their maximum size, i.e. the length of the minor axis, as they were about to leave the pharynx. Another solution was then passed through under the cover-glass for ten minutes, after which measurements were made on another series of successive food-vacuoles in the same specimen or in a different specimen in the same solution. This was repeated with still other solutions. Then the whole process was repeated with other specimens. The results obtained are presented in Tables I and II.

TABLE I

Time required to form food-vacuoles in Vorticella similis

Time in seconds required to form each of seven consecutive vacuoles in each of six individuals, selected at random

	In pond-water			In distilled water		
	a	b	c	d	e	f
	40	48	52	69	79	42
	38	56	51	70	83	58
	51	56	29	50	83	58
	62	50	39	67	86	46
	31	50	39	75	56	54
	39	49	38	43	58	44
	42	46	39	60	?	35
Average	43.3	50.7	41	62	74.1	48.1
Total average	45			61.4		

Results

Table I shows that there was marked variation in the time required for the formation of consecutive food-vacuoles in all six vorticellae studied, that the time required varied much with the individuals under both conditions and that it was on the average much longer in distilled than in pond-water. The results presented demonstrate, therefore, that the rate of formation of food-vacuoles is much higher in pond-water than in distilled water.

Table II shows that the successive food-vacuoles in each of the five specimens tested varied greatly in size in all the solutions used, but that the food-vacuoles

TABLE II

Variation in the size of the food-vacuoles in Vorticella similis *and the effect of various substances on its size*

A, a specimen subjected successively to distilled and pond-water; a, b, c and d, four specimens, each subjected successively to the solutions indicated. The lactose, NaCl and $CaCl_2$ solutions were made with redistilled water and they were equal in osmotic concentration.

All the measurements for each specimen in a given solution were made on successive vacuoles.

	Length in micra of minor axis at maximum size															
	Distilled water	Pond-water	Redistilled water				Lactose 0.026 M				NaCl 0.014 M				$CaCl_2$ 0 01 M	
Designation of specimens	A	A	a	b	c	d	a	b	c	d	a	b	c	d	a	b
No. of vacuoles measured	5	5	8	9	9	9	8	9	9	5	9	6	4	6	5	5
Minimum	12.7	13	11.56	7.14	7.46	8.5	13.6	7.48	6.12	8.84	6.8	5.44	3.4	4.76	6.8	4.76
Maximum	14.1	16	17	10.2	9.18	10.2	17	10.1	8.16	10.88	14.16	7.48	4.76	5.44	8.8	6.12
Average	13.4	14.1	14.6	8.3	8.2	9.9	15.2	8.7	7.3	9.9	11	6.3	4	5.2	7.3	5.4
Total average for a and b			11.26				11.75				9.12				6.35	
Total average			10.12				10.13				7.36					

in different specimens in the same solution and in the same specimen in different solutions varied even more. It shows that in the four individuals measured the average length of the minor axis of the vacuoles ranged from 7.3 to 15.2μ in the solution of lactose, from 4 to 11μ in the solution of NaCl and from 5.4 to 7.3μ in the solution of $CaCl_2$. It indicates that the vacuoles were on an average slightly larger in pond-water than in distilled water, the same in size in redistilled water and the solution of lactose, much smaller in the solution of NaCl, and the smallest in the solution of $CaCl_2$.

Discussion

The osmotic concentration of the solution of lactose used was obviously much higher than that of the redistilled water. The fact that the food-vacuoles formed

in these two fluids were practically the same in size indicates, therefore, that osmotic concentration is not involved in regulating the size of the vacuoles.

The solutions of lactose, sodium chloride, and calcium chloride used were equal in osmotic concentration and in acidity. The differences in the size of the food-vacuoles formed in these solutions were therefore not correlated with either of these two factors. They consequently must have been correlated with the chemical properties of the substances in the solutions.

The hydrogen-ion concentration of the distilled water used was pH 5.5 and that of the pond-water pH 8.2; the osmotic concentration of the latter was much higher than that of the former and they differed greatly in chemical composition. The results referred to above indicate that the size of the food-vacuoles is not specifically correlated with the osmotic concentration or the acidity of their contents. The difference in the size of the vacuoles observed in pond-water and distilled water was therefore not due to either of these two factors. It consequently must have been due to difference in the chemical composition of their contents.

The results in hand seem to show therefore that the size of the food-vacuoles in *Vorticella* is largely, if not entirely, dependent upon the nature of the chemicals they contain.

As stated above the rate of formation of food-vacuoles is higher and the vacuoles are larger in pond-water than in distilled water. The rate of ingestion of fluid is therefore higher in the former than in the latter, but since these fluids differ greatly in acidity, osmotic concentration, and chemical composition, the difference in the rate of ingestion may be due to any one or any combination of these factors.

We are well aware that some of the results presented in this section are equivocal, and that more results are needed before valid conclusions concerning the regulation of the size of the food-vacuoles and the rate of ingestion can be reached. We had intended to extend the observations made and to investigate the effects of other chemicals in various concentrations, but other duties interfered and we see no prospect of continuing the work in the near future. We are therefore presenting these inadequate results with the hope of encouraging further work.

Changes in the Size and the Form of the Food-vacuoles

Introduction

After the food-vacuoles have been separated from the pharynx they move rapidly to the posterior end of the body on a definite course, as previously stated, then slowly on a very indefinite course to the vestibulum. They are spindle-shaped until they reach the posterior end of the body then they usually become spherical and gradually decrease in size to a minimum, remain so for about two minutes and then rapidly increase in size again (Fig. 6).[3] Numerous measurements were made with a stopwatch and an ocular micrometer on the time required for these changes and their extent. The following results were obtained:

[3] During the decrease in size the particles in suspension frequently, but not always, aggregate near the center of the vacuole, leaving a clear space at the surface (Fig. 6), which soon disappears, but usually forms again when the vacuole begins to enlarge, after which the particles soon become equally distributed.

Change in form.—The time required for the change in the form of the food-vacuoles from spindle-shaped to spherical varies enormously. Under some conditions it occurs almost immediately after the vacuoles have reached the posterior end of the body. Under others it requires an hour or more and under still others it probably does not occur at all.

The rate of change in form seems to be closely correlated with the viscosity of the content of the vacuoles. The particles in suspension in the fluid in the vacuoles which changed rapidly in form were invariably in violent Brownian movement, indicating low viscosity, whereas those in the vacuoles which changed slowly were often practically stationary, indicating high viscosity. In specimens which had ingested carmine granules or lactose (0.025–0.05 M) the change was consistently very slow.

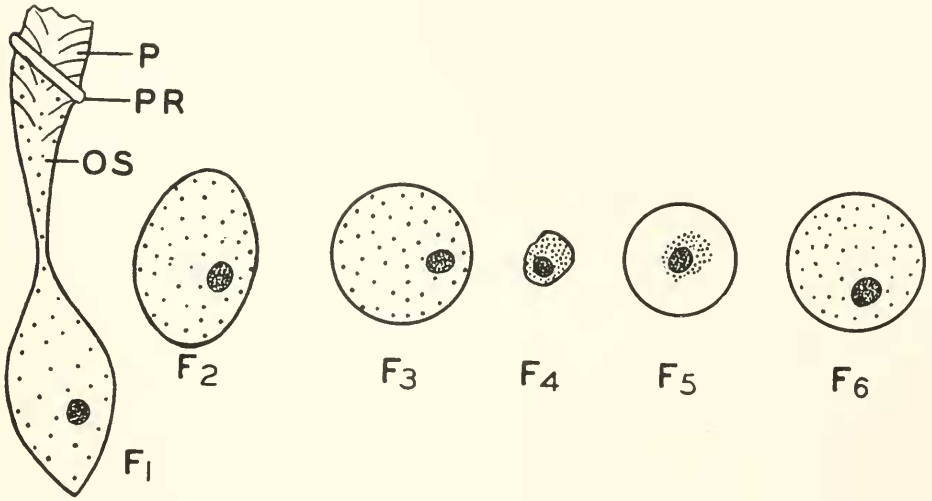

FIGURE 6. Camera outlines showing the separation of a food-vacuole from the pharynx in *Vorticella similis* and subsequent changes in its size and form.
P, pharynx; *PR*, pharyngeal ring; *OS*, oesophageal sac; F_1, a food-vacuole in the process of separation from the oesophageal sac; F_2–F_6, subsequent stages in the food-vacuole.

Decrease in size.—The decrease in size is due to loss of fluid. This ordinarily continues until there is no perceptible fluid left in the vacuoles and the surface membrane is in close contact with the mass of particles. Consequently, if the vacuoles contain relatively few particles, they decrease much more than if they contain many, and if the particles are large, yeast-cells, e.g. the surface becomes very irregular. Many of the vacuoles which were measured decreased three-fourths in diameter, i.e. to one sixty-fourth in volume. Under some conditions there is, however, still considerable fluid in the vacuoles when they have become minimum in size and under these conditions the shrinkage is obviously less. The reduction in size requires from one to three minutes.

The loss of fluid, resulting in the decrease in the size, is probably due in part to difference in the osmotic concentration of fluids in the vacuoles and the cytoplasm

(that of the latter being higher than that of the former)[4] and in part to inward pressure of the elastic membrane on the surface of the vacuoles, which was stretched by the pressure of fluid forced into them by the pharyngeal cilia.

If the decrease in the size of the food-vacuoles is correlated with excess external osmotic concentration, the extent of change in size should decrease if the internal osmotic concentration is increased. This can readily be accomplished by adding physiologically neutral osmotic substance to the surrounding medium.

Observations were consequently made on vorticellae in pond-water containing lactose in various concentrations, and the vacuoles formed in each concentration measured at short intervals after they left the pharynx. It was found that no vacuoles were formed in concentrations higher than 0.05 M, that in concentrations of 0.05 M and lower the vacuoles decreased in size and that they decreased least in the highest of these, i.e. 0.05 M, but that the decrease in this concentration was definitely less than in pond-water, the maximum being not more than one-third in diameter in place of three-fourths or more. It was also found that after the vacuoles had reached minimum size, those formed in the lactose solutions contained much more fluid than those formed in pond-water and were never irregular in shape, like many of those formed in pond-water.

These results show that difference between internal and external osmotic concentration is involved in the observed decrease in the size of the vacuole and they indicate that the inward pressure of the membrane around them is also involved.

If this is true, it is obvious that fluid leaves the vacuoles, not only from the time they reach the posterior end of the body until they have become minimum in size, but continuously from the very beginning of their formation, for these two factors function in the oesophageal sac as well as in the vacuole, since the vacuole is, as previously demonstrated, merely a portion of the sac.

In vorticellae in the lactose-pond-water solution, it was repeatedly observed that the food-vacuoles often coalesce with each other after the sudden increase in size and that owing to this and lack of elimination of undigested substance, the body became well filled with huge vacuoles. Coalescence of vacuoles was not observed in pond-water or culture fluid. The lactose must consequently produce changes in the vacuolar membrane which make it possible.

Increase in size.—The vacuoles usually remain minimum in size for nearly two minutes, then *very rapidly* increase until they are nearly, if not quite, as large as they were originally, after which they remain fairly constant in size until their content is discharged into the vestibulum (Fig. 3).

The increase in size requires on an average, a little less than three seconds. During this time the vacuole is literally flooded with fluid from the cytoplasm. This fluid usually first appears as a well defined layer between the membrane and the viscous central mass, then this mass disintegrates and the solid particles in it soon become uniformly dispersed with violent Brownian movement throughout. Digestive enzymes are doubtless carried from the cytoplasm into the vacuoles with the fluid that enters, for digestion begins soon after the vacuoles have enlarged.

In *Paramecium* and some other ciliates numerous so-called neutral red bodies aggregate on the surface of the food-vacuoles. It is maintained by some that these

[4] The osmotic concentration of the fluid in the cytoplasm, as will be demonstrated in the last section of this paper, is approximately 0.3 atmospheres higher than that of the surrounding medium.

bodies contain digestive enzymes, that they enter the vacuoles and are therefore involved in digestion. In the Peritricha there is no aggregation of such bodies on the food-vacuoles and there is no indication that any enter. They are consequently in all probability not involved in digestion in these organisms.

The inflow of fluid, resulting in increase in size, is probably entirely due to greater osmotic concentration within the vacuole than without. If this is true, the internal osmotic concentration must increase greatly during the time that the vacuole remains minimum in size. This could readily be brought about by transformation in the vacuole of osmotically inactive to osmotically active substance, for example, starch to sugar.

In food-vacuoles which contain lactose, the gelatinous substance in them, referred to above, increases greatly in viscosity as the vacuoles decrease in size (as indicated by observations on Brownian movement) and then decreases greatly as they increase in size. The increase in viscosity is correlated with increase in acidity (as will be demonstrated presently). It may well be that this increase in acidity causes chemical changes in the gelatinous substance which result in increase in osmotic concentration and that this in turn causes the rapid inflow of fluid from the cytoplasm which in turn, owing to decrease in acidity, causes the observed decrease in viscosity.

Changes in the Hydrogen-ion Concentration in the Food-vacuoles in Vorticella

Introduction

Numerous observations have been made by several investigators on the hydrogen-ion concentration in the food-vacuoles in various protozoa. Nearly all the results obtained indicate that as the food-vacuoles pass through the body the hydrogen-ion concentration first increases, then decreases and then remains nearly constant. However, only a few of the observations concern the extent of these changes. Shapiro (1927) on the basis of changes in the color of indicator dyes, concludes that in the food-vacuoles in *Paramecium* the hydrogen-ion concentration increases to pH 4, then decreases to pH 7, that in *Vorticella* it increases to pH 4.5, then decreases to pH 7 and that in *Stylonichia* it increases to pH 4.8, then decreases to pH 7. Claff et al. (1941) using essentially the same methods conclude that in *Bresslaua* it increases to between pH 4.2 and 3 and then decreases (extent not given). Mast (1942) using similar methods and others found that in *Amoeba* it increases to pH 5.6, then decreases to pH 7.3. And Howland (1928) on the basis of results obtained by injecting dyes into the food-vacuoles, concludes that in *Actinosphaerium* it increases to pH 4.3 ± 0.1 and then decreases to between pH 5.4 and 7.

These conclusions indicate that the change in hydrogen-ion concentration in the food-vacuoles differs greatly in the protozoa. The validity of some of them is, however, so equivocal that further investigations are highly desirable. Detailed observations were therefore made on the changes in the hydrogen-ion concentration in the food-vacuoles in *Vorticella*. Two methods were used: one consisted of observations on the solubility of crystals in the vacuoles; the other of observations on changes in the color of ingested yeast-cells which had been stained with various indicator dyes.

Ingested crystals indicating acidity

Neutral red was added to pond-water (pH 8.2) and left for several hours. During this time numerous long needle-like yellowish brown crystals formed. Some of these crystals were broken up by mounting a little of the solution containing them under a cover-glass on a slide and vigorously tapping the cover-glass. Some of the broken crystals were then drawn under the cover-glass on a preparation containing several vorticellae in pond-water. The vorticellae occasionally ingested pieces of the crystals, some minute, others as long as the diameter of the vacuoles. A considerable number of vacuoles containing such pieces were carefully observed. No changes were seen in any of the pieces of the crystals until after the vacuoles which contained them had left the pharynx and had decreased considerably in size (but not to a minimum) then they suddenly dissolved.

The relation between the solubility of these crystals and the acidity of the solution surrounding them was ascertained by adding some to Clark buffer solutions differing in hydrogen-ion concentration and to Hahnert culture solutions containing different quantities of HCl. It was found that their solubility is closely correlated with the acidity of the solutions and that the lowest acidity in which they dissolve readily is approximately pH 5 in the buffer solutions and approximately pH 4 in the HCl solutions (Mast, 1942).

The results obtained in the observations on the crystals in the food-vacuoles indicate, therefore, that the hydrogen-ion concentration of the fluid in the food-vacuoles increased from pH 8.2 to about pH 5 as the size of the vacuole decreased. But since the crystals dissolved before the vacuoles had (as stated above) reached their minimum size, the maximum acidity of the fluid in them must have been higher than pH 5. The results obtained in the following observations confirm this contention.

Ingested indicator dyes showing maximum and minimum acidity in the food-vacuoles

Methods.—Yeast-cells were boiled in distilled water containing respectively the following indicator dyes: meta cresol purple (range pH 1.2–2.8 and 7.4–9), thymol blue (range pH 1.2–2.7 and 8–9.6) metanil yellow (range pH 1.2–2.8), benzopurpurin (range pH 1.2–4), dimethyl yellow (range pH 2.8–4.4), brom phenol blue (range pH 3–4.6), methyl orange (range pH 3.2–4.4), methyl red (range pH 4.2–6.3), brom cresol purple (range pH 5.2–6.8), congo red (range pH 3–5), brom thymol blue (range pH 6–7.6), neutral red (range pH 6.8–8), phenol red (range pH 6.8–8.4), nile blue (range pH 7.2–8.6), and cresol red (range pH 7.2–8.8). The yeast-cells stained well in benzopurpurin, brom phenol blue, congo red, brom thymol blue, neutral red and nile blue but not in any of the others.

Some yeast-cells stained with each of these six different dyes were put respectively into pond-water (pH 8.2) and presented to vorticellae in pond-water under cover-glasses.

In nearly all the preparations the vorticellae ingested some of the stained yeast cells, and in these they sometimes ingested them so freely that the food-vacuoles became well filled with them. The number in the vacuole could, however, be controlled by regulating the number in suspension in the surrounding medium.

Observations were made on numerous vacuoles, containing various numbers of yeast-cells, from the time the cells entered the oesophageal sac until they were discharged.

A series of Clark buffers [5] in small test-tubes was arranged in a row in a test-tube rack for each dye and an appropriate amount of the dye added to each buffer in the series. The acidity of adjoining buffers differed by 0.2 pH. The color of the stained yeast-cells in the vacuoles was, as the vacuoles formed and circulated in the body, continuously compared with those of the buffers in the series containing the dye under consideration and the hydrogen-ion concentration of that which it most nearly matched noted. It is assumed that this was the hydrogen-ion concentration of the substance in the vacuole at the time the comparison was made.

Congo red (pH 3, orange–pH 5, blue)

The results obtained with yeast-cells stained with congo red are more clear-cut than those obtained with any of the other dyes used. This is due to the brilliance and density of the color of the yeast-cells stained with this dye and to the striking change in color correlated with changes in hydrogen-ion concentration.

The yeast-cells in the pond-water in which the vorticellae were mounted were dense brilliant orange in color. Those which were ingested retained this color for an average of 75 seconds after the food-vacuole had left the pharynx, then, as the vacuoles decreased in size, they gradually became purple, then more and more bluish until the vacuoles had become minimum in size and the cells, if there were but a few in a vacuole, sky-blue in color (about pH 3). This color they now retained for an average of nearly 2 minutes, i.e. until the vacuoles very rapidly increased in size, then the cells suddenly became orange of the same shade as that which they had when they entered the vacuoles. This color was retained until the content of the vacuoles was discharged which usually occurred within half an hour. There was no indication of digestion in the discharged yeast-cells. A typical record taken from our notes reads as follows:

(2:10 p.m.) A yeast-cell entered a vacuole; (40 sec. later) the vacuole, containing only one yeast-cell, left the pharynx, spindle-shaped, 12μ long and 8μ wide, yeast-cell still orange; (75 sec. later) yeast-cell slightly purple,[6] vacuole spherical, 8μ in diameter; (75 sec. later) yeast-cell sky-blue (about pH 3), vacuole 3μ in diameter, slightly irregular in form; (2 min. later) yeast-cell orange, vacuole spherical, 8μ in diameter; (15 min. later) yeast-cell orange, no change in structure, vacuole same in size.

The results presented indicate, therefore, that the acidity of the fluid in the food-vacuoles in *Vorticella* increases nearly, if not quite, to pH 3 and that this is closely correlated with decrease in the size of the vacuoles.

The conclusion that increase in the acidity of the content of the food-vacuoles is closely correlated with decrease in size is strongly supported by results obtained in observations on congo red-stained yeast-cells ingested in 0.05 M lactose in pond-

[5] The following buffers were used: phthalate, pH 2.6–3.4; acetate, pH 3.6–5.6; phosphate, pH 5.8–8; borate, pH 7.8–10.

[6] Under high power (oil-immersion objective), it could be seen clearly that the central portion of the cells was still orange and that the purple was confined to a thin layer at the surface.

water. In these observations it was found that the food-vacuoles do not decrease as much in size as they do in pond-water without lactose, there always being considerable fluid left in them and that the color of the yeast-cells usually changes from orange to purple but never to blue, indicating that the acidity of the content of the vacuoles increases only to pH 5 in place of nearly to pH 3.

We repeatedly observed that if the food-vacuoles contained many congo red-stained yeast-cells, the cells did not become blue. We consequently made extensive observations on the relation between the number of yeast-cells in a vacuole and the extent of change of color in them and found the following:

In the vacuoles which contained five cells or fewer there usually was a change in color from orange to blue, but it required considerable longer in those which contained five than in those which contained only one or two cells. In those which contained six to nine cells, there usually was a change from orange to purple but not to blue, and the decrease in the size of the vacuoles was much less than in those which contained only a few cells. In the vacuoles which contained ten or more yeast-cells no change in color was observed and there was but little if any decrease in size. Two typical records from our notes follow:

(10:05 a.m.) A vacuole containing five yeast-cells left the pharynx; (2 min. later) yeast-cells getting purple, vacuole but little larger than the five cells; (1 min. later) cells bluish; (15 sec. later) cells blue, very little fluid in vacuole, irregular in form; (30 sec. later) cells turning orange, vacuole clearly larger, nearly spherical, hyaline layer at surface; (45 sec. later) cells orange, vacuole spherical, original size.

(10:30 a.m.) A vacuole containing about ten yeast-cells left the pharynx; observed continuously for six min.; no perceptible change in the color of the yeast-cells or the size of the vacuole.

These results show that the extent of change in color from orange toward blue in congo red-stained yeast-cells in the food-vacuoles and the extent of decrease in size of the vacuoles vary inversely with the number of yeast-cells in the vacuoles. They consequently support the conclusion that increase in the acidity of the content of the vacuoles is correlated with decrease in their size.

The question now arises as to whether or not the extent of change in color depends upon the time that the yeast-cells are in the vacuoles. Information concerning this question was obtained by making observations on cells which entered the vacuole at different times. Since the formation of the vacuole required from 30 to 60 seconds, this sometimes differs by nearly 60 seconds. It was repeatedly observed, however, that if a yeast-cell enters immediately after the vacuole begins to form it takes just as long for it to become purple after the vacuole has left the pharynx as it does if the cell enters just before the vacuole leaves it. Moreover, several vacuoles were studied in which one cell had entered at the beginning of formation and another just before the end of formation, and it was found that in all these vacuoles the two cells became purple and blue at the same time, although one of them had been in the vacuole nearly 60 seconds longer than the other. These results show that the acidity of the solution in the forming vacuoles is not high enough to have any perceptible effect on the color of yeast-cells and that the observed changes in color in them is not specifically correlated with the time they have been in the vacuoles.

Brom phenol blue (pH 3, yellow–pH 4.6, blue; benzopurpurin pH 1.2, violet–pH 4, red)

The results obtained with brom phenol blue confirm in general those obtained with congo red. With vorticellae in pond-water the stained yeast-cells were dense sky-blue when they entered the food-vacuoles and they became distinctly greenish yellow when the vacuoles had reached minimum size, but their color corresponded more nearly with buffer, pH 3.2 than pH 3. When the vacuoles increased in size the yeast-cells rapidly became blue again. The results obtained with brom phenol blue therefore indicate that the maximum acidity reached by the substance in the vacuoles is pH 3.2, i.e. not quite so high as is indicated by those obtained with congo red.

The yeast-cells stained with benzopurpurin were deep red when they entered the food-vacuoles and no appreciable change occurred as the vacuoles passed through the body. If the maximum acidity in the food-vacuoles is actually pH 3 as the results obtained with congo red indicate, one might expect some evidence of change in the color of the cells stained with this dye, for its range extends from pH 1.2 to 4. The difference in color between buffer pH 3 and pH 4 was however so inconspicuous that it would be extremely difficult to distinguish in yeast-cells in food-vacuoles. The fact then that no change in color was observed in the food-vacuoles containing yeast-cells stained with benzopurpurin, does not seriously militate against the results obtained with congo red and brom phenol blue.

The results presented, therefore, seem to prove that the acidity of the substance in the food-vacuole in *Vorticella* increases from somewhat less than pH 5 to a maximum of pH 3.2 as the size of the vacuole decreases to a minimum and that the acidity very rapidly decreases as the size of the vacuole suddenly increases, and they show that this decrease extends beyond the highest limit of the ranges for the dyes used, namely pH 6.8, but they do not show how far beyond this range it extends. The results obtained with brom thymol blue and neutral red concern this, and also the acidity of the content of the oesophageal sac.

Brom thymol blue (pH 6, yellow–pH 7.6, blue); neutral red (pH 6.8, red–pH 8, amber); nile blue (pH 7.2, blue–pH 8.6, purple)

The yeast-cells stained with brom thymol blue were deep blue when they entered the vorticellae in pond-water. In the oesophageal sac, they became distinctly yellowish, pH 6.4, if there were but few present. After the vacuoles had formed and left the pharynx and began to decrease in size they soon became bright lemon yellow, pH 6; then when they suddenly increased in size they rapidly became yellowish blue, like buffer pH 6.8, possibly pH 7, but positively not so blue as pH 7.2 and not nearly so blue as they were when they entered the vacuoles. The results obtained with brom thymol blue consequently indicate that the minimum acidity reached is approximately pH 6.9.

The yeast-cells stained with neutral red were brownish yellow (pH 8.2) when they entered the vacuoles. They very soon became reddish pink after the vacuoles had left the pharynx and began to decrease in size, but there was no appreciable change in color when the vacuoles later suddenly increased in size. The color of the buffers in the prepared series was essentially the same from pH 5 to pH 7, but at pH 7.2 it was distinctly yellowish. There was no indication of this color in the

yeast-cells in the old vacuoles. The acidity in these cells therefore did not decrease to pH 7.2. These results therefore support the conclusion reached on the basis of those obtained with brom thymol blue, namely, that the minimum hydrogen-ion concentration reached in the substance in the food-vacuoles in *Vorticella* during the process of digestion is between pH 6.8 and 7, i.e. that the substance in the food-vacuole decreases greatly in acidity but does not actually become alkaline.

The yeast-cells stained with nile blue in pond-water were sky-blue and there was no change in color in those which were ingested. These results therefore have no bearing on the problem under consideration.

It can be concluded, then, that in *Vorticella* after the food-vacuole leaves the pharynx, the acidity of its content increases from approximately pH 6.4 nearly to pH 3 in about two minutes, with a decrease in size during this time to about 1/27 of its original volume, that it then remains nearly constant in acidity and in size for nearly two minutes, after which it very rapidly increases in size with a very rapid decrease in acidity to about pH 6.9. The problem concerning the processes involved in the changes in size has been considered in a preceding section; that concerning those involved in the changes in acidity will be considered in the following section.

Discussion

Shipley and DeGaris (1925) maintain that in *Paramecium* the fluid in the food-vacuole first becomes alkaline, then acid, then alkaline again. We obtained no evidence whatever indicating a preliminary alkaline phase in the food-vacuoles of *Vorticella*. Shapiro (1927) also failed to find any indication of it in this genus, but he maintains that he found a preliminary alkaline phase in *Paramecium* if the culture fluid is neutral but not if it is alkaline. It would seem, however, that in alkaline solutions, as Howland (1928) has well said, "it obviously should have been more prominent than in neutral solutions." The contention of Shipley and DeGaris is consequently equivocal. Moreover, evidence will be presented in a subsequent paper which indicates that it is not valid.

The food-vacuoles in *Actinospherium* into which Howland (1928) injected dyes contained active ingested organisms. These, owing to metabolism, undoubtedly caused increase in the acidity of the fluid in the vacuoles and the mechanical injury produced by the pipet used in the process of injection also augmented the acidity. The maximum acidity she observed, namely pH 4.3 $\pm$ 0.1, is therefore higher than that which obtains under normal conditions in vacuoles which do not contain living organisms.

Claff et al. (1941) maintain that in culture fluid containing neutral red, the fluid in the food-vacuole in *Bresslaua* becomes pink and also the organisms in it after they die, indicating increase in acidity. They hold that this increase in acidity is due to "a sudden release of an acid into the newly-formed food-vacuole" from the surrounding cytoplasm. But they also maintain that there are numerous "cherry red granules" in the cytoplasm and that many of them aggregate on the surface of the vacuole. We have made many observations which strongly indicate that the pink color observed by Claff et al. in the fluid was due to the effect of the "cherry red granules" on the transmitted light, not to dye in the fluid, and that the pink color in the dead organisms was due to the acid produced in them as they died, not to acid

in the fluid around them, for similar changes in color occur in organisms which die in neutral red solutions which are not in the food-vacoules.

To obtain accurate results with dyes concerning the hydrogen-ion concentration of the content of the food-vacuoles in protozoa, it is therefore necessary to avoid injuring the cytoplasm around the vacuoles and to consider the effect of colored granules in the cytoplasm on the light transmitted through it, and the acid produced by metabolism and death of organisms in the vacuoles.

In the methods used in the observations on changes in acidity in the food-vacuoles in *Vorticella* considered above all these sources of error were avoided. The results obtained must therefore be fairly accurate.

Factors Involved in the Changes in Acidity in the Food-vacuoles

It is widely held that change in acidity observed in the food-vacuoles in the protozoa is due to secretion of acid or base by the cytoplasm adjoining the vacuoles (Greenwood and Saunders, 1894; Nirenstein, 1905; Lund, 1914; Howland, 1928; Claff et al., 1941). Mast (1942) maintains, however, that this does not obtain in *Amoeba*. He says that in this organism "the cytoplasm secretes neither acid nor base" and he concludes (p. 203): "The increase in the acidity of the fluid in the food-vacuoles probably is due to respiration in the ingested organisms, chemical changes associated with their death, disintegration of the ingested plasmalemma, impermeability to acids of the membrane around the vacuoles and diffusion of fluid from the vacuoles. The decrease in acidity is due to diffusion of alkaline fluid from the cytoplasm into the vacuoles. The cytoplasm secretes neither acid nor base."

Let us consider these views in reference (1) to the increase and (2) to the decrease in acidity observed in the food-vacuoles in *Vorticella*.

(1) *Increase in acidity*

If the increase in acidity in the food-vacuole is due to secretion of acid by the surrounding cytoplasm, the acid must pass from the cytoplasm either into the oesophageal sac or the food-vacuoles. We have demonstrated that fluid passes continuously out of the food-vacuoles from the time they begin to form until they have become minimum in size, i.e. during the time that the acidity in them increases to maximum. Consequently, if the increase in acidity is due to secretion of acid by the cytoplasm, it must pass into the pharynx or the vacuole against an outward current of fluid. This is highly improbable. Moreover, since the acidity of the content of the food-vacuoles reaches pH 3.2 and that of the adjoining cytoplasm is, as will be demonstrated presently, approximately pH 7.4 the acid in the vacuoles could come from the cytoplasm only by active secretion. There is, however, no indication whatever of a structure by means of which this could be accomplished. Secretion of acid by the cytoplasm into the vacuole is therefore not at all probable.

If living organisms in the food-vacuoles are involved in the increase in acidity in them, there obviously should be no change in acidity in food-vacuoles which do not contain living organisms. The following observations concern this:

Vorticellae were mounted in normal pond-water, then this was replaced by sterile pond-water by letting it flow continuously through the preparation for at least

five minutes, then yeast-cells stained with congo red in sterile pond-water were added. The vorticellae ingested some of the yeast-cells and the color of those ingested changed as the vacuoles proceeded on their course. No difference in these changes and those which occur in normal pond-water, either in time or shade, could be detected.

This experiment was repeated several times with sterile pond-water and also with distilled water. The results obtained agree with those presented above, with the exception that in distilled water it required a little less time for the change from orange to purple (increase in acidity) and the vacuoles were not quite so small when it occurred. This is doubtless due to the fact that the distilled water used was pH 5.5 and contained no buffers, whereas the pond-water was pH 8.2 and contained buffers, and therefore required more acid to produce the observed increase in acidity.

It can consequently be concluded that if metabolism in living organisms in the food-vacuoles in Vorticella is a factor in the production of the observed increase in acidity, it is of minor importance.

Yeast-cells which have been stained with congo red are, as previously stated, not digested. In the food-vacuoles formed by vorticellae in distilled water, containing these cells, there is consequently very little if any digestion. It was found, however, that the increase in acidity in these vacuoles is just as great as it is in those which contain an abundance of digestible substance. It is therefore obvious that digestion is not extensively, if at all, involved in the production of acid in the food-vacuoles. What, then, causes the observed increase in acidity in the food-vacuoles?

Lund (1914, p. 14) demonstrated that in *Bursaria* the acidity of the substance which enters the vestibulum increases as it passes thru the pharynx and he concluded that this shows that the cytoplasm secretes acid and pours it into the pharynx. There is, however, a more likely cause of the increase in acidity observed by Lund.

In the protozoa the cilia in the feeding apparatus are very active during the process of feeding and they perform a considerable amount of work in forcing fluid into the vestibulum and through the pharynx into the oesophageal sac. Metabolism in them and in the cytoplasm associated with them is, therefore, high. This, owing to the production of carbonic, lactic, and other acids, causes increases in the hydrogen-ion concentration[7] of the fluid as it passes through the feeding apparatus. After the fluid has entered the oesophageal sac, some of it passes out through the limiting membrane into the cytoplasm and still more after the food-vacuole has been formed and has left the pharynx, as shown by its rapid decrease in size. Moreover, Bozler (1924), Fortner (1924, 1926), Eisenberg (1925), Müller (1932) and especially Frisch (1937) have demonstrated fairly conclusively that fluid passes continuously from the pharynx into the cytoplasm during the process of feeding. This would further increase the acidity of the substance in the pharynx as it passes through, if the wall of the pharynx is impermeable to the acids produced by metabolism but permeable to bases, as it may well be. It is therefore highly probable that the increase in acidity in the pharynx observed by Lund is due to the end products of metabolism rather than to secretion by the cytoplasm.

The acid in the pharynx obviously passes into the food-vacuole, and if the

[7] By adding brom thymol blue to weakly buffered culture fluid containing protozoa, it can readily be demonstrated that they produce acid in the process of metabolism.

membrane at the surface of the vacuole is impermeable to acids but permeable to bases, the acids will remain in the vacuole as fluids and bases pass out, and the acidity of its content will increase. Mast (1942) accounted for the increase in the acidity of the food-vacuole in *Amoeba* by means of similar assumptions. The source of the acid appears however to differ greatly in the two organisms.

The question now arises as to whether the loss of fluid from the food-vacuole in *Vorticella* is great enough to produce the observed increase in acidity in it. We do not know precisely what the hydrogen-ion concentration of the content of the food-vacuole is when it leaves the pharynx, but the results obtained in observations on ingested yeast-cells stained with brom thymol blue indicate, as stated above, that it is about pH 6.4. However, Lund (1914) found in observations on *Bursaria* that ingested vitellin and yolk granules in an alkaline solution containing litmus, change from blue to red in the pharynx, before they enter the food-vacuole. This shows that the content of the forming food-vacuole in *Bursaria* is distinctly acid. Lund has reproduced the color assumed by the litmus-stained granules in the pharynx. By comparing this color with that of litmus paper in each of a series of buffers, ranging from pH 5.2 to pH 6.6, it was found that it is more nearly like the litmus paper in buffers pH 5.8 (and lower) than that in any of the other buffers in the series. This indicates that the solution ingested by *Bursaria* changed from distinctly alkaline approximately to pH 5.8. These results support the conclusion reached above, namely, that the hydrogen-ion concentration of fluid ingested by *Vorticella* increases considerably before the food-vacuole leaves the pharynx, and they indicate that it probably increases to pH 6. If this is true, the acidity of the food-vacuoles in *Vorticella* increases approximately from pH 6 to a maximum of pH 3.2 as the vacuoles decrease in size.

As previously stated, the decrease in the size of the food-vacuoles and the increase in the acidity of their content varies greatly, the one being roughly proportional to the other. Let us therefore consider the results obtained in actual measurements of the changes in size and acidity observed in a typical vacuole. These results show that the vacuole selected decreased in size from an ellipsoid $8 \times 12\mu$ to a sphere 3μ in diameter and that the acidity of its content increased approximately from pH 6 to pH 3.2. If it had been full of fluid at pH 6 when it was maximum in size, it would have contained 6×10^{-19} moles of H^+, and if it had been full of fluid at pH 3.2 when it was minimum in size, it would have contained 89×10^{-19} moles of H^+, i.e., it would have contained nearly 15 times as much H^+ when it was minimum in size as it would have when it was maximum in size. According to the postulated hypothesis it should contain the same amount. The loss of fluid during the reduction in size would therefore not have been sufficient to account for the observed increase in acidity on the basis of this hypothesis. The vacuole was however not full of fluid. It contained approximately three percent of solids when it was maximum in size and 97 percent when it was minimum. It therefore contained about three percent less than 6×10^{-19} moles of H^+ or 5.82×10^{-19} moles at maximum size, and 97 percent less than 89×10^{-19} moles of H^+ or 2.67×10^{-19} moles at minimum size, i.e., less than half as much as at maximum. The increase in the concentration of hydrogen-ions, owing to loss of water during the decrease in the size of the vacuole, would therefore seem to be ample to account for the observed increase in acidity if there is, in accord with our hypothesis, no loss in hydrogen-ions.

(2) Decrease in acidity

The decrease in acidity in the food-vacuoles is, as previously stated, accompanied by a very rapid and extensive inflow of fluid from the cytoplasm. The fact that this inflow requires only about three seconds and is many times as great in volume as the fluid already in the vacuole, indicates very strongly that the decrease in acidity is due to low acidity of the fluid which enters from the cytoplasm, and that nothing in the nature of secretion is involved.

The Hydrogen-ion Concentration of the Cytoplasm in Vorticella

No one has previously investigated the hydrogen-ion concentration of the cytoplasm in any of the ciliates but several have investigated it in the rhizopods. Pantin (1923) maintains that the hydrogen-ion concentration of the cytoplasm in a small marine amoeba is pH 7.6–7.8 in the plasmasol, pH 7.2 in the plasmagel and pH 6.8 in the protruding pseudopods. Needham and Needham (1925) conclude that in *Amoeba proteus* it is pH 7.6 throughout, and Chambers, Pollack and Hiller (1927) contend that in *Amoeba proteus* and *Amoeba dubia* it is pH 6.9 $\pm$ 0.1.

Mast (1942) has considered these contentions critically. He contends that the methods used are not reliable and comes to the conclusion on the basis of his own observations that the hydrogen-ion concentration of the cytoplasm in *Amoeba proteus* is approximately pH 7.4.

The results presented above show that in *Vorticella* the flooding of the food-vacuole with fluid from the cytoplasm usually causes the vacuole to increase about 25 times in volume and the acidity of their content to decrease approximately from pH 3.2 to pH 6.9. But since the vacuole contains approximately 97 percent solids at minimum size and only some five percent at maximum, the fluid in it increases more than 500 times. Since the two fluids mixed in the vacuole are buffered, and their relative amounts and the hydrogen-ion concentration of one of them and that of the mixture are known approximately, that of the other (the fluid in the cytoplasm) can be ascertained approximately, by mixing appropriate buffers in proper proportions and measuring the hydrogen-ion concentration of the mixture. This was done, and it was found that if one part of a pH 3.2 buffer is added to 500 parts of a pH 7 buffer (the approximate proportion of the two fluids mixed in the vacuole), the hydrogen-ion concentration of the mixture is pH 6.98. This indicates that the hydrogen-ion concentration of the cytoplasm in *Vorticella* is slightly higher than pH 7, i.e., considerably higher than that of the cytoplasm in *Amoeba proteus*.

The Function of the Changes in the Hydrogen-ion Concentration in the Food-Vacuoles

Hemmeter (1896), Howland (1928) and Claff et al. (1941) maintain that the increase in acidity in the food-vacuoles in protozoa serves to kill the ingested organisms. Nirenstein (1905) concludes, however, that in *Paramecium* the acidity of the content of the food-vacuoles does not become high enough to kill the ingested organisms and Mast (1942) comes to the same conclusion in reference to *Amoeba*. It is consequently doubtful whether the increase in acidity functions as a killing

agent in any protozoa, and according to Greenwood and Saunders (1894), Nirenstein (1905) and Mast (1942) it does not function directly in digestion in *Amoeba* and *Paramecium,* for digestion does not begin in these organisms until after the acidity in the food-vacuoles has decreased to a minimum.

Vorticella, as previously stated, feeds almost exclusively on bacteria. After the bacteria have been carried into the forming food-vacuole by the action of the cilia in the feeding apparatus, they swim actively about in the fluid in it and they continue swimming until a few moments after the vacuole has left the pharynx and has decreased somewhat in size, then they stop abruptly (all coming to rest at practically the same instant) and usually soon aggregate in a dense mass in the central region of the vacuole. They have doubtless been killed, for they do not become active again when the vacuole enlarges and the mass breaks up. This also occurs if lactose (0.05 M) is added to the culture fluid.

The hydrogen-ion concentration of the fluid in the vacuoles when the bacteria became inactive, could not be accurately measured, but the results obtained in observations on ingested yeast-cells stained with congo red indicate that it is not higher than pH 5. Moreover, in culture fluid containing 0.05 M lactose the acidity, as stated above, increases only to approximately pH 5. The bacteria in it are, therefore, not subjected to higher concentration of acid than this.

The lethal concentration of acid for the bacteria was ascertained by adding to given quantities of culture fluid different quantities of hydrochloric acid and measuring the time the bacteria in the culture fluid lived. It was found that they lived indefinitely in the culture fluid at pH 5 and more than 30 seconds in the culture fluid at pH 4. It is consequently obvious that death of the bacteria in the food-vacuoles is certainly not entirely due to the increase in acidity.

The time between the separation of the vacuole from the pharynx and the cessation of movement of the bacteria in it, was measured with a stopwatch. It was found that this varies considerably in consecutive vacuoles in the same individual, but that the average for different individuals is fairly uniform. The variation for ten consecutive vacuoles in a typical individual was 14 to 18 seconds with an average of 16.3 seconds.

It requires, as stated above, about 50 seconds to form a food-vacuole. The bacteria which enter when it begins to form are, therefore, in it about 66 seconds before they are killed, whereas those which enter just before it leaves the pharynx are in it only about 16 seconds. The cause of death must therefore be due largely, if not entirely, to changes in the content of the vacuole after it leaves the pharynx. There are, as previously stated, two very prominent changes during this time, increase in acidity and decrease in fluid. It was demonstrated above that the increase in acidity is not fatal. Death in the food-vacuoles is therefore probably due to the loss of fluid. Mast (1942) comes to the same conclusion in reference to the cause of death in the food-vacuoles in *Amoeba.* He contends that the loss of fluid augments the decrease in oxygen in the vacuoles due to respiration in the bacteria, to such an extent that it is fatal.

There is no visible indication of digestion of the bacteria in the food-vacuoles until after the acidity in them has decreased to minimum. This seems to show that the increase in acidity does not function in digestion. There are however profound changes in the vacuole while the acidity in it is maximum for, as previously stated, the osmotic concentration of the fluid in it during this time increases greatly. It

may well be, therefore, that the increase in acidity functions in the production of this increase in osmotic concentration, e.g. by hydrolizing complex molecules, which in turn functions in the inflow of fluid-carrying enzymes which facilitate digestion.

The decrease in acidity in the food-vacuoles is clearly correlated with digestion, but since it is merely the result of rapid inflow of fluid from the cytoplasm it is obviously not the result of anything in the nature of secretion by the cytoplasm.

The Osmotic Concentration of the Cytoplasm in Vorticella

If the decrease in the size of the food-vacuoles in *Vorticella* were entirely due to difference between internal and external osmotic concentration, and if the membrane at the surface of the food-vacuoles were permeable to water only, and if no osmotically active substance passes into the feeding apparatus from the cytoplasm, the osmotic concentration of the cytoplasm could be measured by changing that of the ingested fluid until there is no decrease in the size of the vacuole after it leaves the pharynx. It was however demonstrated above that inward pressure of the stretched membrane around the vacuole is functional in the decrease in its size, and it is highly probable that some osmotically active substance enters the feeding apparatus from the adjoining cytoplasm. The decrease in the size of the food-vacuole is therefore probably not closely correlated with the relation between the osmotic concentration of the fluid in the food-vacuole and that of the fluid in the cytoplasm. It was found however that the size of the entire body varies consistently with the osmotic concentration of the surrounding medium and that this relation can be fairly accurately measured. Observations on it were therefore made as follows:

A vorticella attached to a fragment of *Lemna* was mounted in pond-water or tap-water under a cover-glass supported on two parallel ridges of vaseline and the length and width of the body measured by means of an ocular micrometer. Then the water was replaced with a solution of lactose in pond-water or tap-water, left ten minutes and the vorticella again measured. This was now repeated with different concentrations of lactose and with different individuals. The results obtained in reference to length are presented in Table III. The width varied directly with the length, but it also varied with the surface viewed. It was therefore not recorded in the table.

Table III shows that the vorticellae decreased in size in the higher concentrations of lactose used, but not in the lower, and that the decrease varied directly with the concentration, but that it was greater in the vorticellae which had been adapted to pond-water than in those which had been adapted to tap-water. It shows that of the seven individuals adapted to pond-water, five became slightly smaller in 0.0125 M lactose in pond-water and two did not change in size, but that in 0.025 M lactose all became definitely smaller; whereas in the nine individuals adapted to tap-water only two became smaller in 0.0125 M lactose in tap-water and only seven became smaller in 0.025 M lactose. The lowest osmotic concentration which causes any decrease in size, is, therefore, a little lower than that of 0.0125 M lactose in pond-water for vorticellae adapted to pond-water, and a little higher than 0.0125 M lactose in tap-water for vorticellae adapted to tap-water. If, then, the decrease in size in the lactose solutions is due to the difference between internal and external osmotic concentration this difference must be slightly less than the osmotic concentration of 0.0125 M lactose for the vorticellae which have been adapted to pond-

Table III

Relation between the size of Vorticella *and the osmotic concentration of the surrounding medium*

Each specimen used was measured successively in the four concentrations; specimen a, three times in each; b, c and d, twice in each; and the rest once in each. The lengths of a, b, c and d given, are averages.

Designation of specimens	Length of body in micra			
	Concentration of lactose in pond-water			
	0.05 M	0.025 M	0.0125 M	0 M
a	77.50	81.66	86.66	90.83
b	52.50	57.50	63.25	65.00
c	43.75	46.75	48.75	50.00
d	60.00	65.00	71.25	72.50
e	75.00	75.00	80.00	82.50
f	50.00	62.50	70.00	70.00
g	57.50	62.50	70.00	70.00
Total average	59.46	64.41	69.98	71.26
	Concentration of lactose in tap-water			
	0.05 M	0.025 M	0.0125 M	0 M
a_1	70.00	77.00	80.50	80.50
b_1	59.50	66.50	66.50	66.50
c_1	56.00	70.00	70.00	70.00
d_1	52.50	66.50	70.00	73.50
e_1	60.75	73.50	77.00	77.00
f_1	52.50	59.50	63.00	63.00
g_1	63.00	66.50	70.00	70.00
h_1	85.75	91.00	92.75	87.50
i_1	53.25	63.00	63.00	64.75
Total average	61.45	70.38	72.52	72.52

water and slightly more than that of 0.0125 M lactose for those which have been adapted to tap-water.

The osmotic concentration of the pond-water used, calculated from the depression of the freezing point, is at 22° C, equivalent to 0.79 atmospheres and that of the tap-water practically zero. The results presented indicate, therefore, that the lower the external osmotic concentration is, the greater the difference between internal and external osmotic concentration becomes.

The osmotic concentration of 0.0125 M lactose at 22° C is equivalent to 0.3282 atmospheres (International Critical Tables). That of the fluid in the cytoplasm must, therefore, be approximately equivalent to 0.3282 plus 0.79 atmospheres or 1.1 atmospheres in vorticellae adapted to pond-water, but only slightly higher than 0.3282 atmospheres in those adapted to tap-water.

Kitching (1938) concludes that in *Zoothamnium* sp., a freshwater peritrich, the excess of internal over external osmotic concentration is equivalent to that of 0.05 M

sucrose, that is, four times as large as the results we obtained in our observations on *Vorticella*. This difference is much larger than would be expected in organisms which are so nearly alike in structure and habitat. Kitching's conclusion was based on results obtained with specimens treated with cyanide. It may well be, therefore, that it is not valid for specimens under normal conditions.

Mast and Fowler (1935) found that 0.005 M lactose in culture fluid is the lowest concentration which produces a consistent measurable decrease in the volume of *Amoeba proteus*. This indicates that the difference between internal and external osmotic concentration is much smaller in *Amoeba* than it is in *Vorticella*.

Summary

1. The feeding apparatus in the Peritricha consists of a ciliated tube (the outer portion of which is called the vestibulum and the inner the pharynx) and about ten fibers (oesophageal fibers) which are attached to the distal end of the pharynx and extend as a bundle through the cytoplasm nearly to the posterior end of the body. There is no oesophageal tube.

2. The Peritricha feed largely on bacteria but various inanimate particles are also ingested.

3. At the end of the pharynx surrounded by the oesophageal fibers there is a cone-shaped sac (the oesophageal sac) which consists of a membrane probably produced by the interaction between the fluid in it and the cytoplasm around it.

4. The pharyngeal cilia force into the pharyngeal sac culture fluid with particles in suspension and usually gelatinous substance secreted by the peristome, the vestibulum and the pharynx.

5. The sac enlarges and becomes spindle-shaped. Then a portion of it is constricted off to form a food-vacuole.

6. The constriction is probably due to local simultaneous inward pressure of the oesophageal fibers.

7. The food-vacuoles vary greatly in size.

8. Initiation of the constriction in the sac and the size of the food-vacuole formed by it are not specifically correlated with the size of the sac or particles in suspension in the fluid in it or the chemical composition of this fluid, but they are to some extent dependent upon these factors, especially the chemical composition of the fluid.

9. The concentration of particles in suspension in the fluid in the oesophageal sac increases as the sac increases in size. This is largely due to the passage of fluid through the oesophageal membrane into the cytoplasm.

10. After the food-vacuole is formed, it passes rapidly on a fixed course through the cytoplasm to the posterior end of the body and then slowly on a varied course to the anal spot in the wall of the vestibulum. The rapid movement is probably due to waves passing synchronously along the fibers. The slow movement is due to cyclosis.

11. After the vacuole has reached the posterior end of the body it usually becomes spherical in form and gradually decreases greatly in size; as it decreases in size the acidity of its content increases to a maximum of pH 3.2, then it increases very rapidly in size and the acidity of its content decreases to pH 6.9.

12. The hydrogen-ion concentration of the fluid in the cytoplasm is approximately pH 7.

13. The decrease in size requires about two minutes. It is due in part to excessive external osmotic concentration and in part to inward pressure of the stretched membrane at the surface. The increase in size requires about three seconds. It is due to excessive internal osmotic concentration probably caused by chemical changes produced by the increase in the acidity of its content.

14. The increase in the acidity of the content of the vacuole is probably largely due to the production of acid, owing to metabolism in the peristome, the vestibulum and the pharynx and impermeability of the vacuolar membrane to organic acid, resulting in its retention and consequent concentration as the vacuole decreases in size.

15. The decrease in acidity is due to the flooding of the vacuole with fluid from the cytoplasm.

16. The osmotic concentration of the fluid in the cytoplasm of *Vorticella* varies directly with that of the surrounding medium. The former is higher than the latter approximately by an equivalent of 0.0125 M lactose or 0.3282 atmospheres.

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